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Principal component and hierarchical cluster analysis to select natural elicitors for enhancing phytochemical content and antioxidant activity of lettuce sprouts

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ABSTRACT

Sprouts have received increasing attention in recent years because of their potential dietary value. Additionally, the efficient production of sprouts with high levels of phytochemicals and antioxidants is desirable. However, no studies were performed on lettuce sprouts. The phytochemical content and antioxidant activity of lettuce sprouts were studied. Moreover, natural elicitors (chitosan and tea tree) were applied as soaking solution during different times or as exogenous daily spraying during germination in order to enhance the phytochemical content of the sprouts. Data was analyzed using multivariate analysis. Untreated lettuce sprouts presented a significant higher content of total phenolics and flavonoids, and antioxidant activity (through DPPH and TEAC assays) than those reported for mature heads of different green lettuce cultivars. Germination percentage was negatively affected by high elicitors concentrations and long contact times. Based on multivariate analysis, only sprouts daily sprayed with elicitors (tea tree 0.18, 0.27, 0.36% v/v and chitosan 0.25, 0.5, 1.0% w/v) presented a significant higher content of phytochemicals and antioxidant activity than the others. Therefore, exogenous application of chitosan and tea tree can stimulate the biosynthesis of phytochemicals, improving the nutritional value of lettuce sprouts.

1. Introduction

Lettuce (*Lactuca sativa*) is one of the most popular leafy vegetables in the world preferably consumed fresh and in salad dishes. It is of particular interest in nutrition due to its content of antioxidants and phytochemicals including caffeic acid and its derivatives, flavonols, vitamins C and E, chlorophyll and carotenoids (Llorach et al., 2008; Viacava et al., 2014). These compounds are associated to health benefits and several studies have shown the health effects of lettuce consumption in improving the lipid status and preventing tissue lipid peroxidation in rats and increasing plasma total antioxidant capacity and antioxidant levels in humans (Nicolle et al., 2004; Serafini et al., 2002).

The enrichment of phytochemicals in plant-based foods suggests the possibility of improving public health through diet. Germination is an inexpensive and simple method for improving nutritive value and edible sprouts are one of the potentially new functional foods (Zhang et al., 2007). However, despite the nutritional composition of lettuce is well known, few studies have determined the nutritional value of lettuce sprouts. Additionally, the efficient production of sprouts with high levels of phytochemicals and antioxidant activity is desirable. In plants, phytochemicals are induced in response to biotic and abiotic stresses, acting as natural phytoalexins to protect plants against these stresses. Some compounds exhibit elicitor activity and highly induce plant defense phytoalexins, suggesting that treatment of plants with elicitors could be a feasible way to trigger the biosynthesis of bioactive metabolites (Pérez-Balibrea et al., 2011b). In this study, two natural elicitors were chosen to study their ability to stimulate the phytochemicals synthesis in lettuce sprouts: the carbohydrate polymer chitosan (CHI) and the essential oil of tea tree (TT). Chitosan is a natural biopolymer obtained by deacetylation of chitin, the linear polymer of (1–4)-β-linked *N*-acetyl-D-glucosamine, a major component of the shells of crustaceans such as crab, shrimp, and crawfish. Recently, chitosan and its oligomers have attracted notable interest due to their biolog-

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ical activities, including antimicrobial, antitumor, antioxidative, and hypocholesterolemic functions (Bautista-Baños et al., 2006). In addition, recent studies have also revealed that chitosan enhances the yield and quality of soybean sprouts and exhibit elicitor activity in broccoli sprouts (No et al., 2003; Pérez-Balibrea et al., 2011b).

The essential oil of *Melaleuca alternifolia* (tea tree) exhibits broad-spectrum antimicrobial activity. Its mode of action against Gram-positive bacteria, Gram-negative bacteria, and yeasts, as well as the native microbiota of different vegetables has been reported (Alvarez et al., 2015; Goñi et al., 2013b). The chemical composition of TT oil has been well defined and consists largely of cyclic monoterpenes of which about 50% are oxygenated and about 50% are hydrocarbons. Among them, terpinen-4-ol is the main antimicrobial constituent. In recent times, TT has gained reputation as a safe, natural and effective antiseptic and it is currently incorporated as natural preservative in many pharmaceutical and cosmetic products (Alvarez et al., 2015). In this current research we investigated if TT essential oil can stimulate the antioxidant synthesis of lettuce sprouts.

The application of chemometric tools for characterization and quality control of food products has recently become a very active research area. Multivariate mathematical approaches are powerful tools which often permit a relatively simple representation of similarities between samples on the basis of more-or-less complex analytical data. Principal component analysis (PCA) is one of the most popular multivariate techniques because it reduces the dimensionality, compresses the noise and correlates measurements in a simple informational sub-space of the data set. Hierarchical cluster analysis (HCA) is another multivariate technique that uses a defined metric to form clusters sequentially; grouping first the most similar objects which are then merged due to their similarities. HCA is used to reveal the structure residing in a data set and disclose the natural groupings existing between samples characterized by the values of a set of measured variables (Patras et al., 2011).

The hypothesis of this research is that yield parameters and phytochemical content of lettuce sprouts can be increased by treating the seeds with natural elicitors, thereby improving the nutraceutical value and seedling growth. To our knowledge, however, the effect of preharvest treatment of exogenous elicitors on bioactive compounds of lettuce sprouts has not been examined. Therefore, the present study aimed to analyze and compare the effects of chitosan and TT essential oil application on antioxidant status, total phenolic and flavonoid contents and growth parameters of lettuce sprouts based on PCA and HCA analysis to improve the healthfulness of this food product with an added value for consumers.

2. Materials and methods

2.1. Elicitors solutions preparation

Various dilutions of CHI and TT were used in order to determine the ideal concentration for maximum elicitor response. Chitosan solutions were prepared by dissolving chitosan powder (ACOFAR, Mar del Plata, Argentina; 98% deacetylation degree) in lactic acid (LA) 0.7% (v/v) and mixed overnight at 100 rpm in an orbital shaker (TS-1000, Zhejiang, China). Lactic acid was used as diluent for chitosan, because pH lower than 6 is required for its appropriate dissolution (Goñi et al., 2013a).

Tea tree (*M. alternifolia*) essential oil was provided by Nelson and Russell (London, England), which supplies food grade oils. TT was diluted in distilled water and vigorously shaken at 30 °C for 30 min to obtain reasonably stable dispersions.

2.2. Seed treatments and germination

Lettuce seeds (*L. sativa* var. Lores) were obtained from Vilmorin® (La Ménitré, France).

Two experiments were consecutively conducted. In the first experiment, 0.5 g of lettuce seeds were mixed with 30 mL of: distillated water (water control), chitosan (0.01, 0.05, 0.1, 0.5% w/v), lactic acid 0.7% v/v (acid control) and tea tree (0.18, 0.27, 0.36% v/v) in an orbital shaker at 100 rpm during 3, 6, 9, 12, 18 and 24 h. The soaked seeds (50 seeds) were then sown in plastic trays (18 × 14 cm) with two layers of Whatman filter paper #42, adequately moistened with 20 mL of distillated water. Trays were covered with plastic foil to prevent dehydration and incubated in a germination chamber (20–22 °C and 8 h photoperiod) for 7 days. The concentrations of chitosan solutions used in this experiment were selected according with previous studies of seed soaking in different vegetal species (Cho et al., 2008; Goñi et al., 2013a). In the case of TT solution, no literature was available about its application as soaking treatment; thus, concentrations were selected based on its application as preharvest sanitizers on late development stages of Butterhead lettuce (Goñi et al., 2013b).

In the second experiment, solutions of: water (water control), chitosan (0.25, 0.5, 1% w/v), lactic acid 0.7% v/v (acid control) and tea tree (0.18, 0.27, 0.36% v/v) were applied by exogenous spraying to the lettuce seeds. 50 lettuce seeds were placed in plastic trays (18 × 14 cm) with two layers of Whatman filter paper #42. 20 mL of each solution was sprayed at sowing day and then daily during 7 days. Trays were covered with plastic foil and incubated as mentioned previously. The concentrations of chitosan solutions used in the second experiment were selected according with previous studies of exogenous spraying in broccoli (Moreno et al., 2008; Pérez-Balibrea et al., 2011b). Concentrations of TT solutions were selected based on its application as preharvest sanitizers on late development stages of Butterhead lettuce, as mentioned previously (Goñi et al., 2013b).

Lettuce sprouts, containing the hypocotyls and cotyledons, were collected at day 7 after sowing and immediately used in the analysis. Percentage of seed germination was determined according to Barassi et al. (2006) by counting germinated seeds at day 7. Only those seedlings without defects were considered as germinated. In order to determine the absence of defects, each tray was thoroughly inspected with a magnifying glass while germinated seedlings were counted.

2.3. Yield parameters

After 7 days of germination, yield parameters (height and weight of sprouts) were measured. The shoot height above the soil was directly measured using a ruler and analyzing at least 10 sprouts per tray. Fresh weight of sprouts was measured after the plant was cut off from above the filter paper and results were expressed as g/50 seeds.

2.4. Extraction of phytochemicals

1 g of lettuce sprouts from each treatment was homogenized with 10 mL solution of ethanol (80% v/v). The homogenate was sonicated for 30 min and then centrifuged at 8000 rpm for 15 min at 4 °C. The supernatant was collected and the precipitate was extracted again with 10 mL of 80% ethanol, under the conditions previously described. The two supernatants were combined and filtered using Whatman filter paper #1. The final ethanolic extract was stored at −20 °C to be used in the determination of total phenolic content (TPC), total flavonoids content (TFC), and antioxidant activity by DPPH and TEAC methods.